β_2-adrenoceptors are the dominant subtype involved in early muscle regeneration after injury

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Skeletal muscles can be injured by a myriad of insults that can compromise their functional capacity. Regenerative processes are often slow and incomplete, and so developing novel therapeutic strategies to enhance muscle regeneration represents an important research area. We have shown previously that the β-adrenoceptor (AR) signalling pathway plays an important role in skeletal muscle regeneration after injury (Beitzel *et al.*, 2004, 2007), and that transgenic mice lacking both β_1- and β_2-ARs have delayed regeneration following myotoxic injury (Sheorey *et al.*, 2008). In the present study we investigated the contribution of β-AR signalling to early muscle regeneration, to determine the relative contribution of individual β-AR subtypes to muscle repair after injury.

Mice (8-9 weeks) lacking β_1-adrenoceptors (β_1-AR KO), β_2-adrenoceptors (β_2-AR KO), or both subtypes of β-adrenoceptors (β_1/β_2-AR KO), were obtained from The Jackson Laboratory (Bar Harbour, ME, USA). Littermate wildtype mice were used as controls for the β_1-AR KO and β_2-AR KO mice, while control mice for the β_1/β_2-AR KO mice were from a C57BL/6 background, as employed previously (Sheorey *et al.*, 2008). Muscle function was determined by assessing the contractile properties of the *tibialis anterior* (TA) muscle *in situ* (Gehrig *et al.*, 2010). Briefly, mice were anaesthetised (60 mg/kg, sodium pentobarbital, *i.p.*), the right TA muscle was surgically exposed, and the distal tendon was attached to the lever arm of a force transducer, with the knee and foot immobilised. At the conclusion of the experiment the mice were killed by cardiac excision while still anaesthetised deeply.

When muscle function was examined in uninjured TA muscles, both β_2-AR KO mice and β_1/β_2-AR KO mice produced significantly less force than their respective controls ($p < 0.05$), however, TA muscles from β_1-AR KO mice showed no significant deficit in force production. To determine the relative contribution of the individual β-AR subtypes to early muscle regeneration, mice were anaesthetised (ketamine 80 mg/kg and xylazine 10 mg/kg; *i.p.*) and the TA muscle of the right hindlimb was injected with the myotoxin, Notexin (1 μg/ml, *i.m.*) to cause complete muscle fibre degeneration. Mice were allowed to recover for 7, 10 or 14 days, after which TA function was assessed *in situ*. β_1/β_2-AR KO mice produced significantly less force than their controls at 7 days post-injury ($p < 0.05$) but force production had increased to similar levels as control at 10 and 14 days post-injury. Muscles from β_2-AR KO mice showed a similar pattern of force production during regeneration with significantly less force at 7 days but similar force production at 10 and 14 days post-injury, while muscles from β_1-AR KO mice did not exhibit force deficits at any stage during regeneration.

These results suggest that the β_2-adrenoceptor is the dominant β-AR subtype involved in early muscle fibre regeneration. Selective stimulation of β_2-adrenoceptors may therefore be a therapeutic strategy to improve the rate, extent and efficacy of the regenerative process, and may have important implications for other conditions where muscle wasting and weakness are indicated.

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